E. coli heat-shock competent cells

Introduction

Protocol for preparing E. coli heat-shock competent cells from http://www.genomearchitecture.com/protocols/ecoli-heat-shock-competent-cells-preparation.html.

When preparing cells, it is important to keep everything chilled for use. For example, you should pre-chill your pipette tip boxes on ice before using.

Materials

- E. coli strain
- LB medium
- LB plates with proper antibiotic
- 0.1 M Ca2Cl solution (ice cold)
- 0.1 M CaCl2 solution containing 15 % glycerol (ice cold)

Procedure

Competent cells preparation

1. The day before: put CaCl2 solutions at 5 C, inoculate one single colony of your E. coli strain in 5 mL LB medium. Shake at +37°C overnight.

   - When preparing cells; filter solutions before use.

2. Put 1.5 mL eppendorf tubes at -80 C in advance.

3. Subculture at 37 C with shaking till OD600 reaches 0.25-0.3 (about 2 hrs subculture time)

4. Chill the culture on ice for 15 minutes.

5. Separate 100 mL chilled bacterial culture into two 50 mL falcon tubes and centrifuge at 4 C at 4000 rpm for 10 minutes.

6. Discard the supernatant and resuspend the pellet with 40 mL ice-cold CaCl2 solution.

7. Keep cells on ice again for 30 minutes.

8. Centrifuge cells at 4000 rpm at 4 C for 10 minutes.

9. Discard the supernatant and resuspend pellet with 5 mL ice cold 0.1 M CaCl2 solution containing 15 % glycerol.

10. Pipet 50 uL (or 100uL, or 200uL) of cell suspension into -80 C frozen eppendorfs and directly transfer them to -80 C freezer.